Myocardial O₂ level does not limit aerobic metabolism.

Myocardial pO₂ does not limit aerobic metabolism in the postischemic heart

A study on hypertrophic myocardium

Youngran Chung
Biochemistry & Molecular Medicine, University of California
Davis, CA 95616-8635

Correspondence:
Youngran Chung
Biochemistry and Molecular Medicine
UC Davis
One Shields Avenue
Davis, CA 95616-8635
Phone: 530-752-5906
Fax: 530-752-3516
Email: yrchung@ucdavis.edu
Myocardial O₂ level does not limit aerobic metabolism.

**ABSTRACT**

Reperfused hypertrophic hearts are prone to develop reflow abnormalities, which is likely to impair O₂ return to the myocardium. Yet, reflow deficit may not be the only factor determining postischemic oxygenation in the hypertrophic heart. Altered O₂ demand may also contribute to hypoxia. In addition, the extent to which myocardial pO₂ dictates energy and functional recovery in the reperfused heart remains uncertain. In the current study, moderately hypertrophied hearts from spontaneously hypertensive rats (SHR) were subjected to ischemia-reperfusion and the recovery time courses of pH and high energy phosphates were followed by ³¹P NMR. ¹H NMR measurement of intracellular myoglobin assessed tissue O₂ level. The study finds that the exacerbation of hypoxia in postischemic SHR heart arises mostly from impaired microvascular supply of O₂. However, postischemic myocardial pO₂, at least when it exceeds ~18% of preischemic level, does not limit mitochondrial respiration and high energy phosphate resynthesis. It only passively reflects changes in O₂ supply-demand balance.

**New and Noteworthy Statement:**

The current study finds postischemic myocardial pO₂ is a mere reflection of O₂ supply-demand balance, devoid of regulatory role, which implies that intramyocellular O₂ gradient is relatively flat in the intact beating heart. Therefore, postischemic pO₂ not recovering to the preischemic level is not necessarily a predictor of poor prognosis.
Myocardial O₂ level does not limit aerobic metabolism.

Keywords: Myocardial hypertrophy; NMR spectroscopy; Oxygen; pH; Reperfusion

Non-Standard Abbreviations and Acronyms: AP, acidic Pi peak; cf, constant flow; cp, constant pressure; CrP, phosphocreatine; HEP, high energy phosphate; I/R, ischemia-reperfusion; LVEDP, left ventricular end-diastolic pressure; Mb, myoglobin; MbO₂, oxy-myoglobin; pLVH, pressure-induced left ventricular hypertrophy; RPP, rate-pressure-product; SHR-nAP, SHR hearts that do not show any AP signals; SHR-tAP, SHR hearts that show tAP signals; tAP, transient acidic Pi peak
Myocardial O₂ level does not limit aerobic metabolism.

INTRODUCTION

Adequate protection of hypertrophic hearts during cardiac surgery poses an ongoing challenge because the tolerance to ischemia is reduced in pressure-induced left ventricular hypertrophy (pLVH). Increased inflammatory or oxidative stress, depressed high energy phosphate (HEP) recovery and mitochondrial dysfunction have been identified as contributing factors (29, 45, 58). In addition, there have been reports that reperfused pLVH hearts are prone to develop reflow abnormalities (4, 56, 59).

One of the reports on reflow abnormalities came from a 31P NMR study of the spontaneously hypertensive rat (SHR), a genetic rodent model of chronic hypertension (59). In this study, investigators utilized pH-sensitive inorganic phosphate (Pi) resonance as the “real time” monitor of reflow and observed two distinct Pi signals representing two different pH values during reperfusion – one pH value reflecting a swift recovery to preischemic level and the other representing the prolongation of acidosis. Demonstrating the correlation between the extent of persistent acidosis indicated by the postischemic acidic Pi signal (AP) and regional flow assessed by dye injection, the investigators proposed using the 31P NMR AP signal as a noninvasive biomarker of no-reflow (59). Since reflow irregularities will impair O₂ return to the heart, our previous observation in the reperfused SHR heart of decreased tissue pO₂ (15) is then consistent with impaired reflow in pLVH.

Yet, the paths leading to tissue hypoxia are twofold; decreased O₂ supply or increased O₂ demand (23). Coronary flow generally matches the metabolic demands of cardiomyocytes (57). However, following an ischemic episode, a so called “functional ischemia” (43) can result if cellular O₂ demand is increased for repairing of ischemic damages (e.g. restoring ion homeostasis) or due to metabolic inefficiency (e.g. mitochondrial uncoupling). Vascular flow,
Myocardial O₂ level does not limit aerobic metabolism.

even when it is restored to the preischemic level or higher, may fail to meet the increased O₂ demand culminating in tissue O₂ deficit (43).

Postischemic hypooxygenation in SHR heart can then be the consequence of either decreased (vascular) O₂ supply or increased (cellular) O₂ demand or both. Ischemic challenge disrupts cellular ion homeostasis. In that context, it is noteworthy that several studies report ischemia-induced ion imbalance is increased in pLVH hearts. For example, Na⁺ accumulation is intensified in ischemic SHR heart (26). Increased Ca²⁺ overload has been reported in the reperfused pLVH hearts (2). Since Na⁺/K⁺ ATPase and sarcoplasmic reticulum Ca²⁺ ATPase (SERCA) activities require energy expenditure, it seems reasonable to postulate that postischemic pLVH heart’s O₂ requirement is increased, thus leads to tissue O₂ deficit, thus less O₂ will be available for HEP resynthesis and contractile function.

While the reports on pLVH heart’s reflow abnormalities abound, it is currently unknown whether reperfused pLVH heart’s O₂ demand is altered from normal heart and potentially contributes to hypo-oxygenation. If it does, to what extent is the postischemic pO₂ deficit caused by altered O₂ demand and to what extent by impaired vascular supply? Is the depressed pO₂ the underlying cause of impaired energy/contractile function recovery often observed in pLVH hearts?

While it may seem obvious that lower cellular pO₂ recovery should lead to lower HEP/contractile function, the relationship between pO₂ and HEP may not be as straightforward as it appears because a detailed profile of pO₂ within cardiomyocyte remains controversial. Isolated cardiomyocyte (33, 60) and intact myocardium studies (61) have suggested the existence of steep intracellular O₂ gradients and anoxic cellular core in the cardiomyocytes surrounded by normoxic extracellular environment, setting off a notion that restricted intracellular diffusion of O₂ could be a limiting factor in the functioning of high-flux metabolic system such as...
Myocardial O2 level does not limit aerobic metabolism. In that case, any recovery less than 100% restoration of cellular pO2 will lead to the expansion of anoxic core and limit mitochondrial respiration and HEP resynthesis. In words, depressed pO2 will become the cause of depressed mitochondrial respiration.

If, on the other hand, intracellular O2 gradient is relatively flat as has been indicated by other studies (23, 24, 64), mitochondrial respiration may recover even in the presence of falling pO2 as cardiomyocytes presumably possess the reserve capacity to increase O2 gradient providing the compensatory driving force for O2 flux. In that case, cellular pO2 and energetics would hold no causal relationship.

Few studies so far have examined whether a recovery of tissue pO2 to the preischemic level is a necessary condition for HEP and other myocardial recovery. If a 100% recovery of tissue O2 level is not a prerequisite, then what is the minimum threshold pO2 required for HEP/contractility recovery and what does it say about intracellular O2 gradient structure of postischemic myocardium? Does the threshold differ between pLVH vs. normotensive heart?

In the present study, perfused, isolated hypertensive (SHR) and normotensive rat hearts were subjected to ischemia-reperfusion (I/R). Another set of normotensive rat hearts were perfused at varying degree of low-flow as the reference to compare with the hearts subjected to I/R. 31P NMR followed the evolution of recovering myocardial pH, AP signals, and HEP. Intracellular pO2 was measured by interleaved 1H NMR while continuous monitoring traced the heart’s contractile function, mVO2, and global flow to address the following questions: 1) What causes depressed pO2 recovery in the postischemic SHR heart; impaired vascular supply or increased cellular O2 demand? 2) Is there a cause-and-effect relationship between depressed pO2 recovery and depressed HEP/contractile function recovery?
Myocardial O$_2$ level does not limit aerobic metabolism.

**METHODS**

*Heart perfusion and NMR*

Heart perfusion and NMR methods have been previously described in detail (15). Briefly, isolated heart was perfused with Krebs-Henseleit buffer equilibrated with 95% O$_2$/5% CO$_2$, warmed to 36°C and containing (in mM) 118 NaCl, 4.7 KCl, 1.2 KH$_2$PO$_4$, 1.8 CaCl$_2$, 1.2 MgSO$_4$, 20 NaHCO$_3$, and 15 glucose. A latex balloon inserted in the left ventricle monitored left ventricular pressure (LVP) and heart rate via a transducer connected to an oscillographic recorder (Gould Windograf). The rate pressure product (RPP), an index of heart’s mechanical function, was calculated by multiplying heart rate by left ventricular developed pressure (LVDP).

Male SHR rats, 7-9 month old, were purchased from Taconic Farms, Germantown, NY. Age-matched Wistar Kyoto (WKY) rats were the controls. The animals were maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The care and experimental procedures were in accordance with the Institutional Animal Care and Use Committee at UCDavis.

All NMR recordings utilized a vertical bore 400 MHz spectrometer (Bruker AMX). The isolated heart was placed in a 20 mm NMR tube, maintained at 36°C with warm air provided by the variable temperature (VT) unit in the magnet. A 1-3-3-1 binomial pulse suppressed the H$_2$O peak and selectively excited oxy-myoglobin (MbO$_2$) Val E11 (γ-CH$_3$) resonance at −2.8 ppm. MbO$_2$ acquisition was optimized with a 45° pulse angle, 40 ms acquisition time and 8065 Hz spectral width. Six thousand transients were averaged for a typical $^1$H spectrum, requiring 5 min of signal accumulation. The MbO$_2$ signal was referenced to H$_2$O peak at 4.67 ppm at 36°C, which was in turn calibrated against sodium 3-trimethylsilyl propionate-2,2,3,3-d$_4$ (TSP). $^{31}$P NMR spectra were acquired using 60° pulse angle and 2 sec repetition time (90° pulse angle and 20 sec repetition time for fully relaxed spectra).
Myocardial O$_2$ level does not limit aerobic metabolism.

Perfusate lactate concentration was determined in triplicates by a lactate oxidase method (YSI 2700 Bioanalyzer). Myoglobin (Mb) leakage from the heart was assessed by scanning the perfusate sample at 410 – 420 nm (5). Creatine kinase activity in the perfusate was measured by spectrophotometric enzyme assay (25). For creatine kinase and Mb, total leakage was calculated by integrating the measured concentration over the time periods of interest. After the perfusion-NMR experiments, hearts were dried at 80°C for 72 hours and weighed.

**Experimental protocols**

During 20 min of baseline perfusion, all SHR (hypertrophic) and Wistar Kyoto (WKY, non-hypertrophic) hearts were perfused at constant pressure (cp) (100 mmHg head pressure) and coronary flow was measured by collecting the effluent. Subsequently, hearts were subjected to normothermic zero-flow global ischemia for 19 min, followed by reperfusion at 100 mmHg in a subset of hearts. Another subset of hearts were reperfused in the constant flow (cf) mode at each heart’s own preischemic flow rate (11.5 – 13.9 ml/min/g heart) to induce 100% recovery of global flow.

Two repetitions of interleaved $^1$H (5 min)/$^{31}$P (5 min) spectra recorded the basal metabolism for 20 min during preischemic perfusion. During ischemia and the first 15 min of reperfusion, $^{31}$P spectra were acquired every 2.5 min, yielding 14 consecutive $^{31}$P spectra. After 15 min of reperfusion, interleaved $^1$H (5 min)/$^{31}$P (5 min) spectra were recorded for another 20 min.

In the control low-flow experiments (n=6, Sprague Dawley rats, 8-9 month old), hearts were perfused in the constant flow (cf) mode throughout the experiment. The use of Sprague-Dawley strain as the control for SHR myocardial studies (in some cases, preferred over WKY) has been previously reported (14, 47). Before the perfusion experiment, heart weight was
Myocardial O₂ level does not limit aerobic metabolism. Following baseline perfusion at 13-17 ml/min/g heart, inflow rates were decreased to 12 ml/min/g for 20 min, then to one more flow step at 2-10 ml/min/g for 20-30 min. (Flow rates used to generate Figure 5 were ml/min/g heart based on actual heart weight measured after perfusion/NMR experiment, not the heart weight estimated prior to perfusion experiment.) Thereafter, flow rate was reverted to baseline level to confirm viability of the heart. No control low-flow hearts were subjected to zero-flow ischemia. Although the low flow condition is in a sense a low-flow ischemia, in the current report the term “ischemia” (appearing as is, or appearing in pre-ischemic, post-ischemic, etc) refers only to the zero-flow ischemia.

Myocardial pO₂

O₂-ligated Mb (MbO₂) in heart gives rise to distinct ¹H NMR signals that allow measurements of intracellular partial pressure of O₂ (pO₂) (42). Fractional Mb oxygenation, $Y = \frac{MbO₂}{Mb + MbO₂}$ --- (1), was determined from the area of the MbO₂ peak and intracellular pO₂ was calculated from the relation, $pO₂ = [pO₂]_{50} \times \frac{Y}{[1-Y]}$ --- (2), where $[pO₂]_{50} (3.8 \text{ mmHg})$ is the O₂ pressure required to half-saturate Mb (5). Based on the retro-fitting procedure previously described in detail (15), the fraction of baseline MbO₂ was determined to be ~75% of total Mb in both SHR and WKY, yielding calculated pO₂ of 11.7 ± 3.2 mmHg (WKY) and 10.5 ± 3.8 mmHg (SHR) (Errors for baseline pO₂ were estimated by the propagation of error method, see “Error analysis”). The baseline pO₂ in SHR and WKY hearts are thus consistent with rodent heart pO₂ measured by other investigators, 6 – 10 mmHg (7, 55, 62, 68).

Yet, a wide range of mammalian sarcoplasmic pO₂ exists in the literature, from ~3 to ~200 mmHg. Using optical reflectance spectroscopy, Arai and coworkers measured >90% MbO₂ saturation in in vivo pig heart (6). Chen and coworkers also reported >90% MbO₂
Myocardial O$_2$ level does not limit aerobic metabolism. 

saturation (corresponding to pO$_2$ >20 mmHg), based on the lack of $^1$H NMR deoxy-Mb signal in the *in situ* dog heart (8, 13). Other studies measured mitochondrial pO$_2$, ~35 mmHg, directly from intact rat heart using O$_2$-dependent quenching of delayed fluorescence emitted from endogenous protoporphyrin (48). On the lower end of the spectrum, 8-10 mmHg in *in vivo* mouse heart (68) and 3-10 mmHg in the frozen sections of *in situ* mammalian hearts have been reported (23, 65).

In the case of *ex vivo* buffer-perfused heart preparations, optical reflectance studies reported 6.3 mmHg in guinea pig (55) and ~6 mmHg in rat (7) (calculated from ~72% and ~70% MbO$_2$ saturation at 37°C, respectively) whereas an EPR oximetry reported ~198 mmHg in rat heart (21). As such, there appears no easily recognizable pattern in myocardial pO$_2$ level along the spectrum of *in vivo* vs. *ex vivo* or invasive manipulations (such as tissue freezing) vs. intact functioning heart.

The wide-ranging variability may partly stem from disparate animal species as well as detection methodologies. NMR detects the whole heart, EPR measures localized areas where the crystals are imbedded and optical spectroscopy measures primarily the epicardial layer due to its shallow penetration depth (~180 – 600 µm) (6, 55).

In addition, optical and NMR measurements of Mb are characterized by inherent uncertainty in establishing 100% and 0% O$_2$ saturation point because of surface O$_2$ diffusion, collateral circulations (6) and the lacking response of % MbO$_2$ to adenosine-induced maximal vasodilation (7). As such, a non-changing Mb signal at high O$_2$ environment does not provide a strong assurance for non-changing pO$_2$ because Mb signal intensity can remain more or less the same while pO$_2$ continues to increase as Mb-O$_2$ binding approaches saturation (see “Limitation” for further details). The current study therefore determined % MbO$_2$ by retro-fitting the response of MbO$_2$ to graded hypoxia into a rectangular hyperbolic function (described in detail in ref.
Myocardial O₂ level does not limit aerobic metabolism. (15)) to indirectly extract the value of normoxic % MbO₂ while avoiding erroneous end-point calibration.

As stated above, an optical study reported ~72% MbO₂ saturation in the perfused guinea pig heart (55), a result very similar to the current study (~75%). While calculating pO₂ from 72% MbO₂ yielded 6.3 mmHg pO₂ (55), a subsequent mathematical modeling using the same data led to the average myocardial pO₂ of ~223 mmHg (10). The >35-fold higher pO₂ was the outcome of the model’s prediction that myocardial O₂ distribution is skewed toward two extremes – one myocyte population with high MbO₂ saturation and the other group of myocytes experiencing relatively severe hypoxia.

Due to the nonlinear Mb-O₂ binding curve, pO₂ calculated from tissue-average % MbO₂ can differ significantly from pO₂ calculated by averaging individual myocyte’s pO₂. For example, if there are two myocyte populations in equal number, one with 99% MbO₂ saturation and the other with 51%, then the average MbO₂ saturation is 75%. Converting 75% MbO₂ to pO₂ gives 11.4 mmHg (if 3.8 mmHg is used as [pO₂]₅₀). Yet if 99% and 51% MbO₂ are separately converted to pO₂ values (376 mmHg and 3.96 mmHg, respectively) and then averaged, the result will be 190 mmHg.

Frozen tissue measurements (23) showed relatively uniform pO₂ distribution across subepicardium as well as along the length of capillary. If myocardial pO₂ distribution is as uniform as reported by these studies, calculating average pO₂ directly from average % MbO₂ may be justified. As the current study does not embark on a mathematical modeling designed to predict the pattern of heterogeneous pO₂ distribution, we will simply present the pO₂ values converted directly from measured % MbO₂ as most other studies have done so (6, 7, 13, 23, 55, 65). However, given the possibility of substantial pO₂ heterogeneity, the tissue-average pO₂ reported in this paper can be an underestimation (or even an overestimation) of true average pO₂.
Myocardial O$_2$ level does not limit aerobic metabolism.

**Capillary perfusion**

Because Mb is equally NMR-visible regardless of its location within myocardium, $^1$H NMR method reports the tissue average Mb from the whole heart in contrast to Doppler or optical imaging methods that detect signals primarily from the epicardial layer (6, 7, 55). Provided that the information on tissue O$_2$ consumption (mVO$_2$) and capillary pO$_2$ are available, the density of perfused capillaries can be estimated from myocardial pO$_2$ using O$_2$ diffusion models (21). It is then expected that % perfused capillary thus estimated should agree with $^{31}$P NMR assessment of no-reflow. Therefore, $^1$H NMR, combined with $^{31}$P NMR detection of acidic Pi (AP) signals, can potentially offer an opportunity to examine the degree to which microvascular abnormalities contribute to postischemic pO$_2$ depression.

The density of perfused capillary was estimated by adapting Krogh’s O$_2$ diffusion model to represent microvascular perfusion, $p_x = p_o - \frac{m}{d} \left( \frac{R^2}{2} \ln \frac{x}{r} - \frac{(x^2-r^2)}{4} \right)$ --- (3) where the symbols represent intracellular pO$_2$ ($p_x$, measured from myoglobin signal), capillary pO$_2$ ($p_o$, calculated from arterial and venous pO$_2$ (35)), oxygen consumption rate (m, mVO$_2$ of heart), tissue O$_2$ diffusion constant ($d$), $\frac{1}{2}$ of intercapillary distance ($R$), distance from a given intracellular point to the nearest capillary midpoint ($x$), and the radius of the capillary ($r$). The values of $d$ and $r$ were taken from the literature reported values for rodent heart and for simplification of calculation $x$ and $R$ were assumed to be identical (21, 57). The inverse of the square of mean intercapillary distance ($1/4R^2$) reflects the density of open capillaries (21). Postischemic capillary density, termed “capillary reperfusion” in this report, was expressed as the % of preischemic capillary density of each heart.

**Phosphate metabolite analysis**
Myocardial O₂ level does not limit aerobic metabolism.

Quantification of $^{31}$P NMR signals (CrP, Pi, and β-NTP) in absolute concentration (mM) has been previously described in detail (15). Briefly, the left ventricular balloon was filled with 100 mM phenylphosphonate. After collecting the baseline $^{31}$P spectra, the balloon was inflated by 0.04 ml, which corresponds to 4 μmols of phenylphosphonate signal. The change in phenylphosphonate signal intensity was then considered to represent 4 μmols of $^{31}$P nuclei and used to calibrate other $^{31}$P signals in the fully relaxed spectra. A fully relaxed spectrum was obtained only in the beginning of the experiment, followed by partially saturated spectra. Assuming that no significant changes in the longitudinal relaxation time (T1) occur as a result of ischemia (46), the same T1 correction factor was applied for the partially saturated spectra obtained during preischemic, ischemic and postischemic periods.

Intracellular pH (pH$_i$) was evaluated from the Pi peak chemical shift using the relation, $\text{pH} = pK + \log \left( \frac{\delta_A - \delta_0}{\delta_0 - \delta_B} \right)$ --- (4), where pK = 6.9, $\delta_A$ = $\delta_{ppm}$ of H$_2$PO$_4$ at 3.290 ppm, $\delta_B$ = $\delta_{ppm}$ of HPO$_4^{2-}$ at 5.805 ppm, and $\delta_0$ = $\delta_{ppm}$ of observed Pi. Ischemic and postischemic Pi resonances are often composite peaks, therefore, asymmetric. Consequently, the median point of the integrated Pi peak, as opposed to the highest point of the peak, was used to determine the pH$_i$.

Postischemic acidic Pi (AP) signal was defined in this study as the postischemic Pi resonance reflecting pH$_i$ no more than 0.2 pH unit alkaline compared with the end-of-ischemic pH$_i$ of each heart. When AP signal was present in the spectrum, a weighted average $\delta_{ppm}$ of the AP and the main Pi signal (reflecting recovered pH$_i$) was used to calculate overall myocardial pH$_i$ (Figure 3C).
Myocardial O₂ level does not limit aerobic metabolism.

**Error analysis**

Data are presented as means ± standard error (SEM). Statistical significance was determined by the standard Student’s t test (p<0.05). Errors for the computed values were estimated by the propagation of error method based on partial differential calculus.

**RESULTS**

**Anatomy and Physiology**

Left ventricle-to-body weight ratio (LV/BW) of SHR heart was ~45% larger than WKY, a moderate hypertrophy (table 1). WKY rats are typically larger than SHR for the same age group. To rule out the possibility that the larger body size of WKY contributed to the underestimation of LV/BW ratio, data from only the comparable weight ranges of SHR and WKY rats (n=15 total, 400 – 500 gram range) was also analyzed, which showed ~34% increase in SHR (p<0.00001). No change in the right ventricle-to-body weight ratio (RV/BW), no signs of hepatomegaly and pleural effusion indicated the absence of heart failure.

Baseline rate-pressure-products (RPP, an index of heart’s mechanical function) were 21,160 ± 1,557 and 28,310 ± 2,062 mmHg min⁻¹ in WKY and SHR, respectively. Phosphocreatine (CrP) levels were 28.2 ± 0.8 and 32.0 ± 0.5 μmol g⁻¹ dry heart in WKY and SHR, respectively. Both RPP and CrP levels were significantly higher in SHR hearts (p<0.05), showing the characteristic signs of (super)compensated hypertrophy (11, 36). MVO₂ in SHR, on the other hand, was not different from WKY, 25.7 ± 2.0 (WKY) and 27.6 ± 1.1 (SHR) μmol min⁻¹ g⁻¹ dry heart (p>0.05).
Myocardial O$_2$ level does not limit aerobic metabolism.

**Ischemia**

Upon stopping arterial flow, left ventricular developed pressure (LVDP) declined rapidly. At ~4 min from ischemic onset, contractile activity was no longer detected (Figure 1A). At ~7 min, hearts began to develop ischemic contracture which reached the peak at ~16 min of ischemia (Figure 1B). SHR hearts developed significantly higher peak contracture pressure (~68-81 mmHg in SHR vs. ~53 mmHg in WKY, p<0.05).

As expected, zero-flow ischemia induced a rapid decline in CrP and increase in Pi (Figure 2 &3A). CrP decreased significantly more rapidly in SHR (p<0.001); during first 1.3 min of ischemia, CrP decreased by 24 ± 0.8 µmol in SHR and 19 ± 0.2 µmol in WKY. By 3.8 min, CrP was depleted in SHR whereas a residual amount of CrP (~14% of preischemic level) was still present in WKY hearts (Figure 3A). ATP concentration, buffered by CrP hydrolysis, decreased at a slower pace than CrP (Figure 3B). The rate of ATP decline was not significantly different in SHR and WKY. Immediately after the onset of ischemia, the rate of acidification in SHR heart was the same as WKY (Figure 3C). Starting from the second time point (3.8 min), however, the decline in intracellular pH (pH$_i$) began to slow down in WKY whereas it remained significantly faster in SHR. Consequently, ischemic acidosis was exacerbated in SHR hearts.

Although MbO$_2$ data were not collected in the current study during ischemia and early reperfusion (Figure 3D), our previous $^1$H time course study showed that MbO$_2$ signals are no longer detected within <2.5 min of ischemia, i.e. pO$_2$ drops to ~0 mmHg, in both SHR and WKY (15).

**Postischemic Recovery**

Please note, since only the constant flow (cf) reperfusion protocol generated sufficient number of SHR hearts that did not develop any AP signals (SHR-nAP), allowing a comparison
Myocardial O2 level does not limit aerobic metabolism.

with and without AP during reperfusion, Figure 1 & 3 depict only data from the cf reperfusion protocol. Results from constant pressure (cp) reperfusion are included in Figure 5, 6, and 7.

A period of ventricular fibrillation and sporadic bouts of tachyarrhythmia (typically ~10 min) followed the onset of reflow until the normal sinus rhythm contractions resumed. Afterwards, RPP continued to increase until it reached the steady level at ~30 min of reperfusion (Figure 1A). In all hearts, the final recovered RPP level was in proportion with mVO2 recovery leaving the aerobic efficiency, RPP/mVO2, unchanged from preischemic ratio (Figure 1C).

The current study observes transient or persistent AP signals in a subpopulation of reperfused SHR heart, a finding similar to previous reports (11, 59). The definition of persistent vs. transient is somewhat arbitrary – persistent AP signals were defined as AP signals that were continuously detected throughout the entire reperfusion (35 min). Transient AP (tAP) signals were detected only during the first ~10 min of reperfusion.

More than half of cf-reperfused SHR hearts (7 out of 11) showed tAP signals during early reperfusion (SHR-tAP) (Figure 1, 3 & 4). The remaining 4 cf-reperfused SHR hearts did not show any AP signals (SHR-nAP). In contrast, in 5 out of 10 constant pressure (cp)-reperfused SHR hearts, AP signals persisted throughout the entire reperfusion period (persistent AP). Four cp-reperfused SHR hearts developed tAP signals and one cp-reperfused SHR heart did not develop any AP signal. No AP signals, transient or persistent, were detected in WKY, except one cp-reperfused WKY heart.

Upon classifying cf-reperfused SHR hearts into two groups, i.e. SHR-tAP and SHR-nAP, it became clear that the difference from normotensive WKY was more prominent in SHR-tAP, whereas metabolic and physiological responses of SHR-nAP heart fell on an intermediate level between SHR-tAP and WKY (Figure 1 & 3). For example, the final recovered levels of CrP, mVO2, pO2 and RPP were in ascending order from SHR-tAP to SHR-nAP to WKY, although
Myocardial O$_2$ level does not limit aerobic metabolism.

HEP and mVO$_2$ initially recovered rapidly in all three groups of hearts (Figure 1 & 3). CrP recovered to ~104% of preischemic level in WKY hearts, accompanied by a characteristic early reperfusion overshoot (~10%) (20, 39). In SHR-nAP and SHR-tAP, CrP recovered to 90% (p>0.05 vs. WKY) and 59% (p<0.001 vs. WKY) of preischemic level, respectively (Figure 3A).

ATP recovered to ~34% of preischemic level in SHR-tAP and ~40% in WKY/SHR-nAP (p>0.05, SHR-tAP vs. WKY) (Figure 3B). It is well documented that ATP content in reperfused heart remains depressed for hours even when aerobic ATP synthesis is fully restored because adenine, a breakdown product of ATP, diffuses to the extracellular space during ischemia and is washed away upon reperfusion reducing the size of intracellular adenine nucleotide pool (20, 39).

Figure 3C shows that the severity of end-ischemic acidosis was also in ascending order, WKY (pH$_i$ 6.25 ± 0.07), SHR-nAP (pH$_i$ 6.01 ± 0.05) and SHR-tAP (pH$_i$ 5.83 ± 0.02), in which SHR-tAP pH$_i$ was significantly lower than those of WKY (p<0.01) whereas SHR-nAP pH$_i$ was not significantly different from WKY. Although the recovery of main Pi signal (that reflects recovered pH$_i$) in SHR-tAP was as rapid as in SHR-nAP or WKY (Figure 4), Figure 3C shows a delayed SHR-tAP pH$_i$ recovery as it depicts the whole heart pH$_i$ derived from the weighted average δ$_{ppm}$ of AP and the main Pi signal.

Similar to CrP, postischemic mVO$_2$ and pO$_2$ in SHR-tAP were significantly depressed compared to WKY (p<0.05), but not in SHR-nAP (SHR-nAP vs. WKY, p>0.05) (Figure 3D). Due to the sensitivity limitation of NMR measurements, early reperfusion time course data were collected for either $^{31}$P or $^1$H, but not both in the same heart. As a result, Figure 3D depicts late reperfusion $^1$H data (pO$_2$); the connecting lines to early reperfusion are the inferences based on our previous $^1$H time course experiments (15) which showed that postischemic pO$_2$ reaches the steady level at least by 1.3 min of reperfusion. Although the current $^{31}$P time course experiments
Myocardial O₂ level does not limit aerobic metabolism.

and the previous ¹H time course experiments were acquired from different groups of hearts, they are parallel sets of experiments (i.e. identical ischemia-reperfusion protocol using same animal models) except that the previous ones followed ¹H time course instead of ³¹P time course.

Disparate responses to ischemic challenge in SHR subpopulations, observed in the current study, have previously been reported. Golden and colleagues (26) reported increased Na⁺ accumulation in ischemic SHR hearts, which correlated with delayed functional recovery. Yet, they noted a subpopulation of SHR hearts unchanged from WKY. Although most of the parameters measured in the current study were nominally depressed in SHR-nAP compared with WKY, none of the differences between SHR-nAP and WKY reached statistical significance. As such, SHR-nAP behaved almost like WKY while SHR-tAP grossly underperformed (Figure 1, 3 & 7), indicating that AP signals, even when they appear only briefly, can predict a poor prognosis in the reperfused heart.

Flow Recovery

Global coronary flow of cp-reperfused hearts recovered to ~72% and ~78% of preischemic flow in SHR and WKY, respectively (p>0.05, SHR vs. WKY) (Figure 5). On the other hand, % capillary reperfusion estimated by Krogh’s O₂ diffusion model (see “Methods”) was ~58% and ~90% of preischemic level in SHR and WKY, respectively (p<0.05). To distinguish microvascular perfusion from global flow, in a subsequent set of experiments, hearts were reperfused in cf mode, which normalized the global flow to 100% of preischemic level in all hearts. Cf reperfusion improved capillary reperfusion in both SHR and WKY (Figure 5). Yet despite 100% global flow recovery, SHR heart’s capillary reperfusion was still depressed compared with WKY (82% vs. 116%, p<0.05). Thus, independent of global flow recovery, SHR microvascular reperfusion was depressed by 32-34% compared with WKY. Finally, while no cf-
reperfused SHR hearts showed persistent AP signals, 50% of cp-reperfused SHR hearts did (Figure 6 & 7).

The degree of tissue edema, indicated by wet-to-dry heart weight ratio, was similar between WKY and SHR (p>0.05, table 1) and was also similar between cp- and cf-reperfused hearts (p>0.05, Data not shown; table 1 shows combined cp/cf data). This indicates that cf reperfusion, despite its propensity to increase perfusion pressure, did not exacerbate tissue edema.

DISCUSSION

Global-microvascular flow mismatch in SHR

SHR heart’s capillary reperfusion was significantly depressed compared with WKY or control hearts at given global flow rates, presenting a clear example of redistribution of total flow and suggesting a substantial portion of global flow was shunted away from microvascular network in SHR (Figure 5). The microvascular reperfusion deficit observed in SHR is likely to originate from functional and structural alterations in coronary vasculature reported in pLVH, which include reduced coronary reserve, luminal narrowing of microvessels, reduction of hyperemic responses, and microvessel compression due to increased diastolic pressure (12). Indeed in the current study the role of diastolic pressure seems paramount; SHR heart’s constant flow-induced increase in capillary reperfusion correlates closely with improved myocardial relaxation (Figure 7B).

According to the vascular waterfall hypothesis, when the microvessels partially collapse and intramyocardial pressure exceeds venous pressure the driving pressure for flow is not
Myocardial O$_2$ level does not limit aerobic metabolism.

arterio-venous pressure difference, but arterial pressure minus intravascular pressure at the collapse point (30). Left ventricular end-diastolic pressure (LVEDP) of postischemic SHR heart was markedly increased over WKY (Figure 1B, 7A & 7B), which implies that effective (waterfall) perfusion pressure must have been decreased in SHR, contributing to lower capillary reperfusion.

While it is well accepted that the incidences of subendocardial no-reflow are increased in pLVH hearts, some studies have shown that the recovery of total flow is also depressed in the postischemic pLVH myocardium (28, 56). Yet, it is unclear whether inducing a recovery of global flow will necessarily lead to the proportionate improvement in microvascular perfusion because vascular resistance at various levels of microvasculature can be redistributed during ischemic stress and the pressure increase accompanying the augmentation of flow can paradoxically provoke autoregulatory vasoconstriction via myogenic mechanisms (17, 52). The potential opposing effect of myogenic response would be especially germane to pLVH hearts as pathological increases in myogenic vasoconstriction have been documented in hypertension (34).

In the intact heart, flow-induced vasodilation is mediated by interaction of various mechanisms, including passive dilation of arteries, increase in mVO$_2$ and consequent metabolic vasodilation or activation of shear stress-sensitive receptors of vascular endothelium (17, 32). While it would be difficult to delineate clearly the individual mechanism in an intact heart study (32), it is still possible to examine whether augmenting global flow and flow-induced vasodilation, regardless of its mechanism, can improve microvascular reperfusion of pLVH heart.

Figure 5 shows that capillary reperfusion increased in response to constant flow (cf) reperfusion (i.e. inducing 100% recovery in global flow) without exacerbating tissue edema in both SHR and WKY (see the account on tissue edema in “Results” section). This implies that
Myocardial O$_2$ level does not limit aerobic metabolism. Flow-induced vasodilation remained intact and prevailed over myogenic constriction in both SHR and WKY despite a general notion that coronary flow should autoregulate at >80-100 mmHg (11). Control low-flow data indeed agree with the expectations of autoregulation; capillary perfusion of the control hearts reached a plateau at ~12 ml/min global flow, which corresponds to ~85 mmHg perfusion pressure and a further increase in flow (and accompanying increase in pressure) did not increase capillary perfusion (Figure 5). The seemingly extended flow response of reperfused SHR and WKY hearts may originate from the metabolic and vascular conditions peculiar to postischemic hearts, which include increased extracellular adenosine and increased activity of endothelium-dependent shear stress mechanotransduction (32, 40).

To what degree the improvement induced by cf reperfusion in SHR was due to flow-induced vasodilation or due to increased waterfall pressure is difficult to discern from the current study. It is likely that these two factors interacted to form a positive feedback loop – flow-induced vasodilation initiates the increase in tissue perfusion and HEP recovery, which in turn reduces diastolic dysfunction raising the waterfall pressure, which further improves microvascular perfusion. While the underlying mechanism remains obscure, the current result implies a potential clinical utility of artificially augmenting flow when resuscitating pLVH hearts, especially pLVH hearts with diastolic dysfunction.

O$_2$ supply and O$_2$ demand both contribute to depressed pO$_2$ in SHR

$^{31}$P NMR indices of reflow abnormalities, i.e. transient or persistent AP signals, are in good agreement with % perfused capillary calculated from $^1$H NMR signals of MbO$_2$ (Figure 6A & 7B). Cellular pO$_2$ level, in turn, correlates with these flow parameters (Figure 6A), consistent with the scenario that impaired vascular supply limits pO$_2$ recovery in SHR. Yet Figure 6A also
Myocardial O₂ level does not limit aerobic metabolism.

shows clearly that pO₂ values in postischemic hearts are significantly lower than pO₂ in control low-flow hearts receiving the same amount of capillary perfusion. Considering flow is the only experimental parameter that was manipulated, therefore assuming flow must be the major determinant of pO₂ in control hearts, the additional pO₂ decrease observed in reperfused SHR and WKY should then arise from factors other than flow limitation.

Analyzing flow vs. non-flow components of pO₂ depression shows that only the non-flow mechanism contributes to postischemic hypoxia in cf-reperfused WKY heart (WKY-cf) (Figure 6B). This is not surprising because WKY-cf hearts were not flow-limited, in terms both of global flow and capillary reperfusion (Figure 5). What was unexpected was the depth of hypoxia in WKY-cf (Figure 3D & 6A), which otherwise showed a full recovery in flow, energy (HEP) and function (RPP). In SHR hearts, on the other hand, microvascular flow limitation plays more dominant role and the relative contribution of flow (relative to non-flow mechanism) linearly increases as reflow abnormalities become more severe (Figure 6B). Therefore, although non-flow mechanism does contribute significantly to pO₂ depression in SHR, the exacerbated tissue hypoxia (compared with WKY) is caused mainly by flow limitation, contradicting the initial hypothesis that abnormally increased O₂ demand exacerbates postischemic hypoxia in SHR.

The relative hypoxia of postischemic heart is consequently expressed as the increased mVO₂/pO₂ ratio (Figure 6C); postischemic mVO₂ in both SHR and WKY hearts were significantly increased from control heart’s mVO₂ at given pO₂. Because of <100% capillary flow recovery, the absolute quantities of postischemic mVO₂ are lower than preischemic level in all heart groups except WKY-cf. The increased mVO₂/pO₂ ratio, however, is an indication that mVO₂ exceeds vascular O₂ supply and results in O₂ supply-demand imbalance. Plotting the flow-independent pO₂ depression vs. mVO₂ increase (over control low-flow hearts) reveals that indeed almost all flow-independent pO₂ depression can be explained by increased O₂
Myocardial O$_2$ level does not limit aerobic metabolism. Consumption (note the regression line intersecting the origin, Figure 6D). The implication of this observation becomes clear; the postulated cause-and-effect relationship (low pO$_2$ recovery → low mitochondrial respiration) does not hold in the postischemic hearts examined in the current study. Instead, the data support the converse; increased respiration causes lower pO$_2$.

**Role of pO$_2$ in myocardial recovery**

An overshoot in capillary reperfusion in postischemic WKY heart (Figure 5 and 7B) is reminiscent of reactive hyperemia and paradoxically increased O$_2$ consumption frequently observed in the reversible ischemia-reperfusion models (18). Consistent with the minimal irreversible ischemic damage, cardiac parameters of cf-reperfused WKY heart – mVO$_2$, contractile function (RPP) and CrP – fully recovered. Yet cellular pO$_2$ did not recover to the preischemic level even in WKY-cf (Figure 3D & 6A). It is unlikely that mVO$_2$-pO$_2$ mismatch in WKY-cf is the result of local regions of focal ischemia interspersed with regions experiencing unusually high O$_2$ consumption, possibly caused by locally increased O$_2$ demand or wasteful O$_2$ consumption via mitochondrial uncoupling. In that case, not only the cellular pO$_2$ but also HEP would have failed to recover fully, which was not the case.

WKY’s mVO$_2$ and CrP that recovered >100% in the presence of depressed pO$_2$ recovery demonstrates that myocardial oxidative metabolism can operate at full capacity without repaying the O$_2$ debt incurred during ischemia. Although most emphatically demonstrated in WKY-cf, the increased mVO$_2$/pO$_2$ ratios (compared with control hearts) are observed in all postischemic hearts indicating that postischemic mitochondrial respiration is not limited by intracellular O$_2$ availability, at least when cellular pO$_2$ is >1.9 mmHg (Figure 6C). As such, the current data cast postischemic myocardial pO$_2$ as a passive reflection of the balance between O$_2$ supply and
Myocardial O2 level does not limit aerobic metabolism. In fact, the lower cellular pO2 of postischemic heart might be an advantage as it can facilitate O2 extraction.

This indicates that extra- or intracellular signaling(s) other than O2 (e.g. Ca^{2+}, phosphorylation potential, NADH/NAD ratio, etc) are involved in raising postischemic mVO2 above preischemic level (WKY-cf) or above low-flow control hearts (all heart groups). Since \( K_m,O_2 \) of cytochrome oxidase is extremely low (~0.1 mmHg) (61), it may indeed be “O2 flux”, not sarcoplasmic “O2 concentration”, that renders a limiting condition for cellular respiration in myocardium.

As a result, the current data implies that intracellular O2 gradient may not be as steep as previously postulated (33, 60, 61). Even at ~1.9 mmHg pO2 (intracellular O2 gradient, if it exists, must be very shallow at this level of pO2), postischemic myocytes were able to muster an extra O2 flux beyond the level achieved by control hearts (~160% increase, Figure 6C). Although observed specifically in the reperfused SHR and WKY hearts, the apparent variability in mVO2-pO2 relationship suggests more generalized phenomenon, that is, the resistance to intracellular O2 transport appears to be low and myocardial respiration is not limited by cellular pO2 possibly except under severely hypoxic conditions approaching ~0.1 mmHg, the level at which mVO2 will eventually be restrained by diminishing perimitochondrial O2 gradient.

These signs of relatively flat intracellular O2 gradient in the contracting myocardium are similar to the findings of near-uniform O2 distribution in isolated cardiac and skeletal myocytes (23, 24, 64) and low critical pO2 for VO_{2max} (~0.5 mmHg) reported in the frozen canine gracilis muscle (24). Indeed, given the postulated role of Mb’s facilitated diffusion of O2 in red muscle, O2 flux in heart could be maximized when intracellular pO2 is relatively low, i.e. within the functional range of Mb-O2 dissociation curve (31, 66). The conclusions reached by previous investigations utilizing isolated myocytes, isolated mitochondria and mathematical deductions
Myocardial O$_2$ level does not limit aerobic metabolism. (16, 23, 24, 31, 66) – cellular respiration is likely to be insensitive to pO$_2$ within wide range of pO$_2$ values – seems to be valid in the adequately reperfused as well as flow-limited postischemic myocardium.

---

**Ischemic acidosis and diastolic dysfunction**

Ischemic acidosis was exacerbated in SHR hearts, especially those that showed transient or persistant AP signals during reperfusion (Figure 3C and 7A). The increased ischemic acidosis in SHR heart has also been observed by other investigators (59) and is likely to originate from increased myocardial glycogen content and/or metabolic alterations; several animal models of pLVH show a decrease in fatty acid oxidation and a compensatory increase in glycolysis (3, 27). Increased lactate accumulation during ischemia, reflected in the first postischemic measurement of perfusate lactate level (24.8 ± 1.3 µmol/min/g dry in SHR-tAP and 10.5 ± 0.8 in WKY, p<0.001), further supports the possibility of increased anaerobic glycolysis in SHR. A delayed ischemic contracture buildup in SHR-tAP (Figure 1B) is also consistent with increased glycolytic ATP production.

Adverse effects of ischemic acidosis are well documented. Ischemic acidosis can directly injure myocytes via acid activation of lysosomal hydrolases (20). It can also indirectly induce postischemic dysfunction by exacerbating cytoplasmic Ca$^{2+}$ overload; H$^+$ clearance during reperfusion can lead to a cascade of ion exchanges involving Na$^+$ influx via Na$^+$-H$^+$ exchanger with subsequent net flux via reverse mode Na$^+$-Ca$^{2+}$ exchanger activity, culminating in Ca$^{2+}$ influx into the myocytes (39, 50).

One of the detrimental consequences of Ca$^{2+}$ overload is myocardial stiffness and diastolic dysfunction. Reperfused SHR hearts showed pronounced diastolic dysfunction in proportion to the degree of H$^+$ accumulation (Figure 7A), consistent with the possibility that
Myocardial O$_2$ level does not limit aerobic metabolism.

Increased ischemic acidosis led to posts ischemic Ca$^{2+}$ overload. Indeed, previous studies reported increased posts ischemic Ca$^{2+}$ overload in aortic constricted pLVH hearts (2).

Diastolic dysfunction can also be induced by ATP deficiency via rigor formation (41). In fact, cellular [Ca$^{2+}$] and [ATP] are interrelated because Ca$^{2+}$ metabolism is partly regulated by ATP-dependent pumps at the sarcoplasmic reticulum and sarcolemmal membrane. Yet, the possibility of ATP deficit contributing to SHR heart’s diastolic dysfunction seems low. In all heart groups, posts ischemic ATP recovered to the similar level whereas the severity of diastolic dysfunction varied widely (Figure 3B, 7A and 7B).

This leaves ATP-independent Ca$^{2+}$ overload as the most plausible mechanism. The close correlation between ischemic acidosis, diastolic dysfunction and capillary reperfusion observed in the current study is intriguing and necessitates a further investigation to establish a causal relationship between the H$^+${ Na$^+$}Ca$^{2+}$ cascade and reperfusion abnormalities in SHR. Indeed this could potentially explain why the contribution of increased O$_2$ demand (in causing depressed pO$_2$ recovery) is less prominent in SHR despite the apparent need for increased energy expenditure. Ion imbalance, while calling for higher O$_2$ demand, will also impede reflow, which then sets the upper limit on maximum O$_2$ supply available for SHR heart despite increased needs.

**Limitations**

In the current study, insulin was omitted because increased insulin resistance in heart and skeletal muscle has been reported in SHR (1, 19). In the absence of insulin, high glucose in perfusate facilitates glucose transport as GLUT1 depends mainly on concentration gradient. However, recent studies have shown high glucose (>$10$ mM) can induce vasoconstriction in isolated arteries and cultured vascular cells (51). Although previous perfused heart studies that used $15$ mM glucose (without insulin) (22) showed no significant differences from the hearts
Myocardial O₂ level does not limit aerobic metabolism.

perfused with 10-11 mM glucose (without insulin) (37, 38, 53) in terms of mVO₂ (~37 vs. 29-35 µmol/g dry/min in hearts perfused with 15 mM vs. 10 mM glucose), ATP (~25 vs. 24-26 µmol/g) or the ability to increase heart’s workload (4.2-fold vs. 3.1-fold increase in RPP when hearts were paced at 600 beat/min with 80 ng/ml dobutamine and EDP raised to 17 vs. 24 mmHg), there remains a concern that glucose-induced vasoconstriction might have influenced the results of the current study. If the relatively high glucose (15 mM) used in the current study predisposed hearts to vasoconstriction, however, there are no indications that SHR heart was disproportionately affected – preischemic MbO₂ saturation was ~75% in both SHR and WKY and SHR hearts showed normal coronary flow and (supra)normal cardiac performance (RPP & HEP) during baseline perfusion.

While fatty acid (FA) is a preferred substrate for myocardial metabolism under normoxic conditions in vivo, there have been reports that FA metabolism is compromised in pLVH heart. Specifically in SHR, both FA uptake and metabolism were shown to be depressed (1, 14, 27). As the current study inquired whether exacerbated tissue hypoxia contributes to SHR heart’s ischemic susceptibility, it was deemed that perfusion conditions that will disadvantage SHR metabolism, such as insulin or FA, will complicate data interpretation. In addition, while typically ~30% of energy is derived from glucose under normal conditions, the dependence on glucose increases during hypoxia, ischemia and pressure overload hypertrophy (44). As the current study focuses on ischemic and postischemic hearts, glucose as the sole substrate was considered adequate for the purpose of the study. Nevertheless, a euglycemic substrate mixture, for example, 5 mM glucose with insulin, ~1 mM lactate/0.1 mM pyruvate, <1 mM fatty acid would be closer to physiological conditions, therefore, should be considered as the substrate choice in the follow-up studies.
Myocardial O₂ level does not limit aerobic metabolism.

The sensitivity limit of \(^{1}H\) NMR MbO₂ method is estimated to be \(~0.2\) mmHg with 5 min signal accumulation, which corresponds to \(~5\%\) of maximum MbO₂ signal intensity (15, 42). Because of the nonlinear nature of Mb-O₂ binding, however, the measurement error becomes magnified at high pO₂. At 90% MbO₂ saturation, a 5% signal detection error leads to \(~100\%\) error in pO₂ calculation (calculating pO₂ from 90% and 95% MbO₂ yields 34 mmHg and 72 mmHg pO₂, respectively). Above \(>95\%\) MbO₂ saturation, a 5% error translates to an inability to distinguish pO₂ ranging from 72 mmHg and higher, practically capping the upper limit of \(^{1}H\) NMR MbO₂ method to \(~70\) mmHg. The limitation applies to both optical and NMR measurements of Mb. As a result, comparative studies employing alternative methods, for example, porphyrin fluorescence lifetime technique (48, 49), will be helpful when examining higher pO₂ ranges.

It is known that coronary flow is not uniform across myocardium and shows temporal fluctuations (9). Perfusion heterogeneity may increase further in the ischemic hearts (54), as has been corroborated by AP-developing SHR hearts in the current study. While this can complicate the interpretation of data, it will not fundamentally affect the analysis because the perfusion heterogeneity, Q/tissue volume (Q: blood flow) can be correlated one-to-one with VO₂/tissue volume (63). As long as (Q-VO₂-pO₂) relationship remains unchanged throughout myocardial volume units (which is a reasonable assumption), the tissue-average measurement should reflect (Q-VO₂-pO₂) characteristics of unit volume.

Reactive hyperemia is likely to be more robust \textit{in vivo} than in perfused heart as the endothelial function of buffer perfused heart may be less than optimum (67). As a result, O₂ supply-demand imbalance and resultant tissue O₂ deficit may be less pronounced \textit{in vivo}. However, the robustness of reactive hyperemia should not change the conclusion arising
Myocardial O2 level does not limit aerobic metabolism. principally from altered mVO\textsubscript{2}/pO\textsubscript{2} ratio and the comparison of postischemic hearts with control low-flow hearts, which remains valid regardless of the absolute value of pO\textsubscript{2}.

Conclusion

The current study finds: 1) In both SHR and WKY, increased O\textsubscript{2} demand contributes to postischemic hypoxia. However, a further exacerbation of tissue hypoxia in SHR, as compared with WKY, arises mainly from microvascular reflow limitation. 2) Myocardial pO\textsubscript{2} in postischemic heart does not regulate oxidative phosphorylation but only passively reflects O\textsubscript{2} supply-demand balance. While restoring O\textsubscript{2} flux is crucial for postischemic recovery, pO\textsubscript{2} per se (at least when it exceeds >1.9 mmHg) does not limit mitochondrial respiration and HEP resynthesis. Apparently, extra- or intracellular signaling(s) other than O\textsubscript{2} elicits the overshoot in O\textsubscript{2} consumption in the reperfused heart. 3) Myocardial oxidative metabolism recovering to preischemic states without fully repaying O\textsubscript{2} debt contradicts the existence of anoxic cellular core and steep intracellular O\textsubscript{2} gradient. The data supports a relatively flat intracellular O\textsubscript{2} gradient. 4) Postischemic heart’s ability to increase O\textsubscript{2} flux at low cellular pO\textsubscript{2} further suggests that the resistance to intracellular O\textsubscript{2} transport is low. 5) Even a brief appearance of $^{31}$P NMR AP signal during early reperfusion can predict a prolonged hypoperfusion and poor prognosis of reperfused heart.
Myocardial O$_2$ level does not limit aerobic metabolism.

**ACKNOWLEDGEMENTS**

I thank Drs. Steven E. Anderson and Fitz-Roy Curry for critical reading of the manuscript.

**SOURCES OF FUNDING**

This work was supported by American Heart Association, Grant-in-Aid 9960023Y (YC).

**DISCLOSURES**

None declared.
References


Myocardial O$_2$ level does not limit aerobic metabolism.


Myocardial O₂ level does not limit aerobic metabolism.


Myocardial O$_2$ level does not limit aerobic metabolism.

**FIGURE CAPTIONS**

**Figure 1**  A. RPP time course.  B. Left ventricular end-diastolic pressure (LVEDP) time course.  C. Cardiac aerobic efficiency.  (SHR-tAP, SHR hearts that showed transient AP signals during early reperfusion; SHR-nAP, SHR hearts that did not develop any AP signals during reperfusion.  Shown in the parentheses are the numbers of animals for each group.)  RPP were normalized to the preischemic level as 100%.  Figure 1 and 3 are based on the data from cf reperfusion experiments.

**Figure 2**  $^{31}$P NMR and $^1$H NMR spectra.

Spectra from a cf-reperfused SHR heart.  CrP resonance was referenced as 0 ppm, which relegates $\beta$-ATP, $\alpha$-ATP, $\gamma$-ATP, intracellular Pi, extracellular Pi peaks to -16.1, -7.5, -2.4, 4.9, and 5.2 ppm.  Note that $^{31}$P signals acquired during ischemia and early reperfusion (2.5 min signal accumulation) need to be scaled up 2-fold (random noise level by a square root of 2) to be comparable to the steady state signals (5 min acquisition) recorded during baseline perfusion (1st row) and the last 20 min of reperfusion (last row).

**Figure 3**  Time course graphs of:  A. CrP.  B. ATP.  C. pH.  D. pO$_2$/mVO$_2$.  (SHR-tAP, SHR hearts that showed transient AP signals during early reperfusion; SHR-nAP, SHR hearts that did not develop any AP signals during reperfusion.  Shown in the parentheses are the numbers of animals for each group.)  Whole heart pH$_i$ was derived from the weighted average $\delta_{ppm}$ of AP and the main Pi signal (reflecting recovered pH$_i$).  MVO$_2$ data are depicted in lines only (devoid of symbols).  Because of the dead space clearance, mVO$_2$ data obtained during the first 5 min of reperfusion are not reliable, therefore, are shown simply as the connecting lines (— — — —).
Myocardial O2 level does not limit aerobic metabolism.

CrP, ATP and mVO2 were normalized to the preischemic level as 100%. Figure 1 and 3 are based on the data from cf reperfusion experiments.

**Figure 4** Four consecutive postischemic \(^{31}\)P NMR spectra (SHR-tAP heart).

Spectrum A was acquired immediately after reperfusion. Extracellular pH (pHo) initially recovers to ~7.28 (with some pH dispersion). By the third spectrum (C), which corresponds to 7.5 min from the start of reperfusion, extracellular Pi peak sharpens and shows the complete recovery to pHo = 7.40. The linewidths of intracellular Pi resonances (A:72 Hz, B:65 Hz, C:95 Hz, D:78 Hz) are significantly increased from preischemic linewidth, 30 Hz (The first row of Figure 1 shows a representative preischemic spectrum.)

**Figure 5. Capillary perfusion vs. global flow.** Data were normalized to the preischemic measurements. 100% global flow in the control low-flow hearts (peak flow beyond which capillary perfusion levels off) correspond to 11.8 ml/min/g heart. Unlike Figure 1, 3, 6, & 7 in which SHR results were further classified into subgroups based on the presence or absence of AP signals, cf- and cp-reperfused SHR data in Figure 5 were plotted without those distinctions.

**Figure 6. Relationship between pO2, mVO2 and capillary reperfusion.** (nAP: no AP, tAP: transient AP, pAP: persistent AP, SHR-cf: cf-reperfused SHR, SHR-cp: cp-reperfused SHR) A. **Cellular pO2 vs. capillary reperfusion.** PO2 and % reperfused capillary were normalized to the preischemic measurements. B. **% Vascular contribution to postischemic hypoxia.** The decrease in postischemic pO2 caused by flow limitation (estimated from the linear regression of control low-flow heart data) was expressed as % of total pO2 decrease from preischemic level. C. **Myocardial O2 consumption vs. cellular pO2.** MVO2 was normalized to the preischemic
Myocardial O₂ level does not limit aerobic metabolism.

D. Flow-independent pO₂ decrease vs. mVO₂ increase. Flow-independent pO₂ depression was estimated from the difference between pO₂ of control low-flow hearts and pO₂ of reperfused SHR/WKY hearts at comparable capillary perfusion level. Relative increase in mVO₂ was the difference between mVO₂ of reperfused SHR/WKY hearts and mVO₂ of control low-flow hearts at comparable pO₂ level. Control values were derived from best-fit lines depicted in figure 6A & 6C.

Figure 7 Relationships between intracellular [H⁺], diastolic dysfunction and capillary reperfusion. (nAP: no AP, tAP: transient AP, pAP: persistent AP, SHR-cf: cf-reperfused SHR, SHR-cp: cp-reperfused SHR) A. Postischemic LVEDP vs. ischemic acidosis. Ischemic acidosis is expressed as the molar concentration of intracellular H⁺ immediately prior to reperfusion. Among cf-reperfused hearts, LVEDP of SHR-tAP was increased from WKY (p<0.05) whereas SHR-nAP heart’s LVEDP was not statistically different from WKY. Among cp-reperfused hearts, both SHR-tAP (p<0.05) and SHR-pAP (p<0.001) hearts showed significantly increased LVEDP, compared with WKY. B. Capillary reperfusion vs. LVEDP. Capillary reperfusion is expressed as % of preischemic capillary perfusion.
Myocardial O$_2$ level does not limit aerobic metabolism.

Table 1. Anatomy and Protein Release Data

<table>
<thead>
<tr>
<th></th>
<th>WKY (n=13)</th>
<th>SHR (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (BW), g</td>
<td>603 ± 18</td>
<td>411 ± 6*</td>
</tr>
<tr>
<td>LV/BW ratio, g dry/g ($\times10^4$)</td>
<td>3.86 ± 0.09</td>
<td>5.58 ± 0.07*</td>
</tr>
<tr>
<td>RV/BW ratio, g dry/g ($\times10^4$)</td>
<td>1.17 ± 0.04</td>
<td>1.24 ± 0.02</td>
</tr>
<tr>
<td>Wet-to-dry heart weight ratio</td>
<td>4.98 ± 0.09</td>
<td>4.94 ± 0.05</td>
</tr>
<tr>
<td>Creatine kinase leakage, Unit g$^{-1}$ heart</td>
<td>68.4 ± 7.7</td>
<td>93.2 ± 12.6</td>
</tr>
<tr>
<td>Mb leakage, nmole g$^{-1}$ heart</td>
<td>35.2 ± 5.9</td>
<td>70.8 ± 13.3</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

* SHR values are significantly different (p<0.05) from WKY.
Fig. 1.

A

Ischemia Reperfusion

RPP, %

WKY (6)
SHR-nAP (4)
SHR-tAP (7)

min

B

Ischemia Reperfusion

LVEDP, mmHg

WKY SHR-nAP SHR-tAP

C

preischemic
postischemic

RPP/mVO2, mmHg/mmol

WKY SHR-nAP SHR-tAP
Fig. 2.

31P

1H

Final Spectra
Reperfusion
Ischemia
Baseline

5 -5 -10 -15 ppm

-2.0 -2.5 -3.0 -3.5 ppm
Fig. 3

A

Ischemia  Reperfusion

CrP, %

min

B

Ischemia  Reperfusion

ATP, %

min

C

Ischemia  Reperfusion

pH

min

D

Ischemia  Reperfusion

pO₂, mmHg

MVO₂ % preischemic

min
Fig. 4

Extracellular Pi

Intracellular Pi

AP

ppm
Fig. 5.
Fig. 6

A

B

C

D

\[ y = \frac{252x}{20 + x}, \quad r = 0.94 \]

\[ r = 0.78 \]
Fig. 7.

A

![Graph A](image)

B

![Graph B](image)